Autophagy 8:10, 1540–1541; October 2012; © 2012 Landes Bioscience

Macroautophagy can press a brake on presynaptic neurotransmission

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The mechanistic target of rapamycin (MTOR) has been implicated in regulating synaptic plasticity and neurodegeneration, but MTOR's role in modulating presynaptic function through autophagy is unexplored. We studied presynaptic function in ventral dopamine neurons, a system from which neurotransmitter release can be measured directly by cyclic voltammetry. We generated mutant mice that were specifically deficient for macroautophagy in dopaminergic neurons by deleting the *Atg7* gene in cells that express the dopamine uptake transporter. Dopamine axonal profiles in the mutant dorsal striatum were ~one third larger in the mutant mice, released ~50% more stimulus-evoked dopamine release, and exhibited more rapid presynaptic recovery than controls. Rapamycin reduced dopamine neuron axon profile size by ~30% in control mice, but had no effect on macroautophagy deficient axons. Acute rapamycin decreased dopaminergic synaptic vesicle density by ~25% and inhibited evoked dopamine release by ~25% in control mice, but not in the *Atg7* deficient mutants. Thus, both basal and induced macroautophagy can provide a brake on presynaptic activity in vivo, perhaps by regulating the turnover of synaptic vesicles, and further regulates terminal volume and the kinetics of transmitter release.

Keywords: macroautophagy, MTOR, dopamine, neurotransmission, striatum, ATG7, 5-hydroxydopamine, vesicles, voltammetry

Submitted: 06/06/12

Revised: 06/27/12

Accepted: 07/02/12

http://dx.doi.org/10.4161/auto.21330

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Punctum to: Hernandez D, Torres CA, Setlik W, Cebrián C, Mosharov EV, Tang G, et al. Regulation of presynaptic neurotransmission by macroautophagy. Neuron 2012; 74:277–84; PMID:22542182; http://dx.doi.org/10.1016/j.neuron.2012.02.020

The master regulatory kinase known as mechanistic target of rapamycin (MTOR) is well known to regulate both protein synthesis and macroautophagy-dependent degradation of cytosolic components. MTOR activity has also long been implicated in regulating synaptic plasticity and neurodegeneration. While a role for MTOR in synaptic plasticity by enhancing protein translation has been reported in multiple studies, MTOR's potential role in modulating presynaptic function through macroautophagy remains unexplored.

To investigate this possibility we chose to study presynaptic function in ventral dopamine neurons. The advantages of this system are that neurotransmitter release and reuptake can be measured directly by cyclic voltammetry independently of postsynaptic response, and that the corticostriatal slice preparation provides a system in which functional dopaminergic terminals can be isolated from somatodendritic regions for up to 10 h. We generated mutant mice (*Atg7* DAT Cre) deficient for macroautophagy by crossing a line that expressed Cre recombinase under the control of the dopamine transporter (DAT) to another line with a floxed *Atg7* gene, rendering autophagy deficiency specifically in dopaminergic neurons.

Under electron microscopy, we found that tyrosine hydroxylase (TH)-labeled dopamine axonal profiles in the dorsal striatum of 2 mo old DAT Cre and *Atg7* DAT Cre mice possessed a similar number of dopaminergic synaptic terminals, but that the axonal profile area was ~one third larger in the mutant mice. Rapamycin-induced MTOR inhibition in vivo caused a ~30% reduction in TH profile size in control DAT Cre mice, but had no effect in *Atg7* DAT Cre animals. Thus, both basal and induced macroautophagy control dopaminergic axonal profile volume in the striatum.

As dopaminergic terminals in the striatum compose only a small fraction

of the total number of terminals in this area, we used the false neurotransmitter 5-hydroxydopamine (5-OHDA) to selectively label dopamine synaptic vesicles. Dopaminergic synaptic vesicle density was decreased by ~25% with acute rapamycin treatment in wild-type and DAT Cre mice, but not in *Atg7* DAT Cre mice, consistent with a role for macroautophagy in the regulation of dopaminergic vesicular contents.

Consistently, evoked dopamine release as measured by cyclic voltammetry was ~50% higher in *Atg7* DAT Cre corticostriatal slices than in DAT Cre controls. Dopamine release was decreased by ~25% with rapamycin in control animals, but not in the *Atg7* DAT Cre mutants. The *Atg7* DAT Cre mutants also exhibited a more rapid recovery of evoked dopamine release. Thus, both basal and induced macroautophagy can place a brake on presynaptic activity in vivo, perhaps by regulating the turnover of synaptic vesicles.

These conclusions were supported by the observation that wild-type rapamycin-treated corticostriatal slices exhibited a transient increase in LC3-II, a phenomenon characteristic of macroautophagic flux. Rapamycin further increased the number of profiles that contained autophagosome-like vacuoles and decreased their total area and the total number of synaptic vesicles.

Altogether, our results demonstrate that macroautophagy plays multiple roles in the presynaptic function of dopaminergic terminals in the striatum, including the turnover and degradation of synaptic vesicles and regulation of terminal volume and the kinetics of transmitter release. An important future goal will be to elucidate the mechanisms by which macroautophagy determines the fate of synaptic vesicle membrane following fusion with the plasma membrane. These membranes, following subsequent endocytosis, are either pointed toward recycling to produce new synaptic vesicles or to degradation, perhaps by utilizing different adaptor proteins during endocytosis.

We also note that rapamycin induced presynaptic macroautophagy generally, producing more striatal synaptic profiles with autophagosome-like vacuoles and decreasing synaptic terminal area and the number of synaptic vesicles in both dopaminergic and nondopaminergic terminals. Thus, our study opens the door for future research on the role of macroautophagy in basal and induced modulation of MTOR in overall synaptic structure and function, and suggests further reasons to explore its role in diseases of the nervous system.